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Thesis abstract

Pluripotent stem cells (PSCs: ESCs and iPSCs) provide an excellent model system for studying neural development and function. These cells also serve as a reliable source of cell replacement for the treatment of various neurodegenerative diseases and disorders. In view of these applications of PSCs, multiple protocols have been developed to direct their differentiation into neural lineage. However, many of these protocols are limiting in terms of (a) low efficiency of generation of neural cells after long-term culture, (b) requirement of exogenous factors to induce and enhance neural differentiation and (c) supplementation of PSC culture medium with serum. Therefore, in the present study, attempts were made to achieve enhanced efficiency of neural differentiation of PSCs in the absence of exogenous molecules by employing a defined culture medium containing knockout serum replacement (KSR). KSR-based culture system was tested with our in-house-derived EGFP-transgenic 'GS-2' ES-cell and 'N9' iPS-cell lines and the wild-type 'D3' ES-cell line. In KSR medium, PSC-derived EBs predominantly generated neural cells from their post-attachment outgrowths and the complexity of neural networks increased as the culture progressed. Molecular phenotyping of PSC-derived neural cells was performed based on the expression of neural markers both at the mRNA and protein levels. qPCR analysis revealed the expression of markers corresponding to multiple neural cell types, including glutamatergic, GABAergic, cholinergic, serotonergic and dopaminergic neurons, astrocytes and oligodendrocytes, at various time points during the culture. RNA expression studies were confirmed via immunocytochemical analysis of the expression of neural markers. On day 15 of culture, FACS quantitation revealed the efficient generation of NES^+ neural progenitors (~16-18%), $MAP2^+$ mature neurons (~12-26%) and $GFAP^+$ astrocytes (~30-63%) from the three PSC lines. Functional assessment of the generated neurons was performed by electrophysiological analysis of passive (RMP) and active (threshold, amplitude, FWHM and outward and inward currents) membrane properties. In order to investigate the role of default pathway in neural differentiation of PSCs in KSR medium, various strategies were employed. GS-2 ES-cells were cultured in the presence of different serum-free supplements; predominant differentiation into neural lineage was achieved in the B27-supplemented medium. The supplementation of KSR medium with BMP4 failed to show any effect of neural differentiation of GS-2 ES-cells. Also, EBs were switched between KSR- and FBS-supplemented culture conditions on day 2 or day 5 of culture. These experiments indicated that KSR medium promoted the generation of neural cell fates at the expense of differentiation to non-neural lineages. Interestingly, differentiation of P19 EC-cells in KSR medium also resulted in the predominant neural differentiation. These experiments collectively suggested the importance of default pathway in neural differentiation of PSCs in KSR medium. Additionally, efforts were made to enrich PSC-derived neural cells and also to enhance the efficiency of neural differentiation of PSCs. The removal of central EB-core from its peripheral neural outgrowth via scooping resulted in the enrichment of neural cells by ~1.3-2.1 folds. Significant increases were observed in the percentages of GS-2 ES-cell-derived $MAP2^+$ mature neurons and $GFAP^+$ astrocytes. Also, FGF2 supplementation of KSR medium was tested as a strategy to achieve enhanced efficiency of neural differentiation. Preliminary studies suggested an increase in the percentage of NES^+ neural progenitors in the presence of FGF2. Taken together, KSR-based culture system offers a simple, defined and efficient method to achieve neural differentiation of PSCs in short time duration in the absence of exogenous factors. KSR-based culture system can be employed to generate specific neural cell types, study molecular regulation of neural differentiation and in disease modeling. Also, it can be used to develop a platform for high-throughput screening of potential neurogenic molecules and for dissecting their mechanisms of action.